

# DNA barcoding cleans house through the Carychiidae (Eupulmonata, Ellobioidea)\*

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**Abstract:** The terrestrial, ellobioid taxon, Carychiidae provides an excellent case study for testing integrative taxonomy and addressing the plethora of historical species designations based on vague morphological characters. Since the Carychiidae are ephemeral, hermaphroditic microgastropods (< 2mm) inhabiting permanently moist aphotic zones of epigeal (*Carychium* O. F. Müller, 1773) and subterranean habitats (eutroglabiont *Zospeum* Bourguignat, 1856), studies investigating their reproductive system for species delimitation have been futile. Many carychiid species designations were established during the mid-18<sup>th</sup> to 20<sup>th</sup> Century heydays of species discovery. Naturalists either split (“splitters”) species by recognizing them according to trivial differences in shell morphology or grouped them (“lumpers”) based upon common morphological traits. The concept of phenotypic variability was not considered or greatly underestimated in these species hypotheses. Although integration of DNA barcodes has since enabled a reliable identification and delineation of most of the traditional morphospecies, still many morphologically unrecognized evolutionary lineages have been found in apparently widespread and variable taxa. In a retrospective, morphometric approach (*i.e.*, starting from genetically identified, DNA-barcoded material), historically established conchological characters of the outer shell of two European *Carychium* species, *Carychium minimum* O. F. Müller, 1774 and *Carychium tridentatum* (Risso, 1826), are investigated. Revealed was a continuum of intraspecific conchological variability, indicating wide areas of overlap between both taxa. The conventional practice of separating species based on shell morphology alone was sufficient for a qualitative species assignment (*i.e.*, when characteristic phenotypes are observed). Molecular analyses, however, enabled a quantitative species assignment for sympatric populations, intermediate morphotypes and juveniles.

**Key words:** phenotypic variability, integrative taxonomy, cryptic evolutionary lineages, canonical discriminant analysis, synonymies

## ECOLOGY, DISTRIBUTION AND DIVERSITY

Reliable taxonomic assignments have a tremendous impact on the exploration, documentation, and interpretation of biological diversity. Consequently, correct species identification is of paramount importance. Since the phenotype is the first impression we perceive about an organism, its identification is reliant on widely applied and established taxonomic morphospecies concepts. Still, modern-day taxonomy and systematics can take advantage of hindsight. By synergistically integrating independent, but complementary lines of evidence, information for the re-evaluation of century-old species hypotheses can be obtained (Dayrat 2011, Haszprunar 2011). Since the diversification of two evolutionary lineages represents a continuous process, all attributes consulted to infer their delimitation (*e.g.*, behavior, ecology, genotype, and phenotype) should manifest variable degrees of differentiation (de Queiroz 2007). Here,

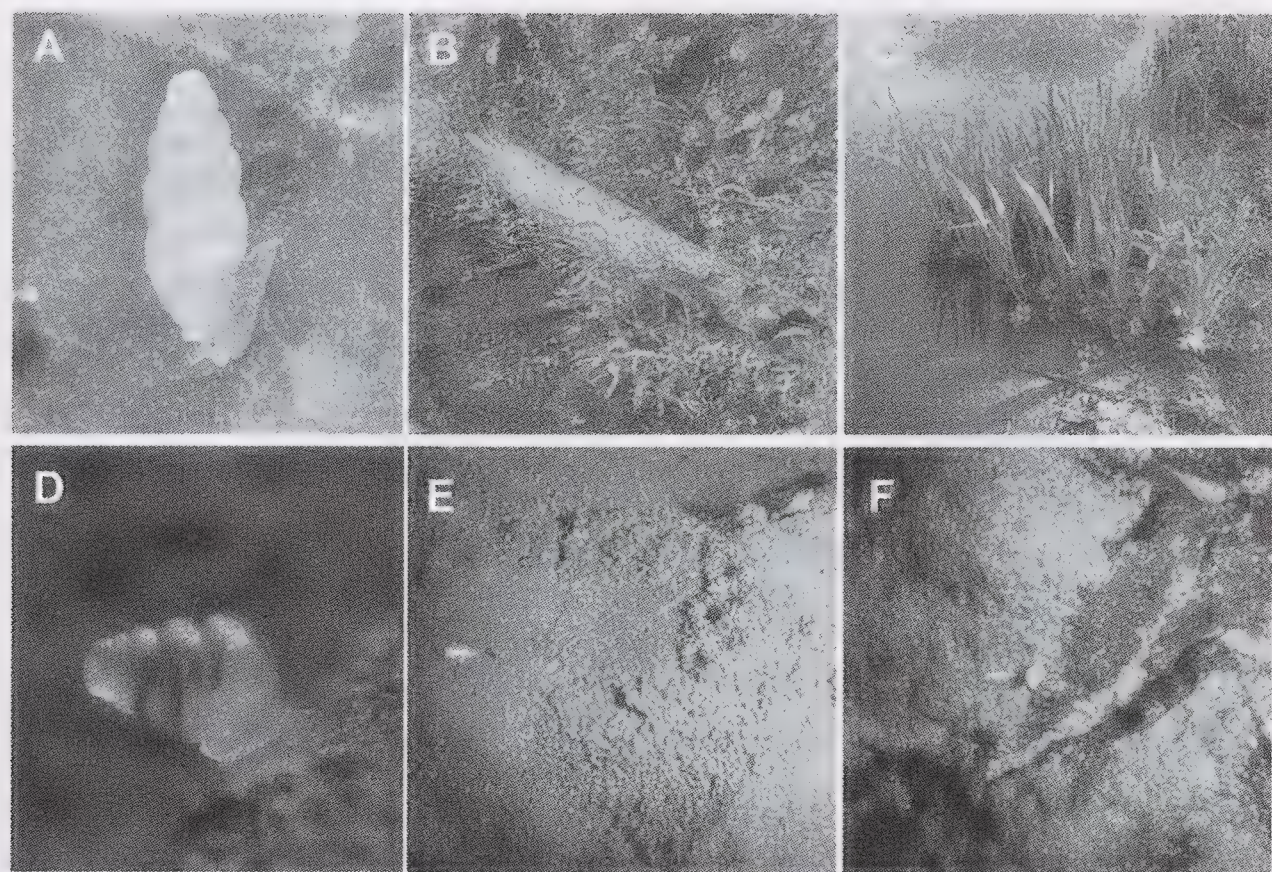
we present a case history of the conchologically-driven microgastropod taxon Carychiidae (Eupulmonata, Ellobioidea) and initiate a new approach for an updated, integrative taxonomy.

The Carychiidae inhabit moist aphotic zones in leaf litter and rotting wood in fens, mesic forests, and riparian zones (epigeal *Carychium* O. F. Müller, 1773) or moist walls and crevices in karst caves (eutroglabiont *Zospeum* Bourguignat, 1856) (Fig. 1). While *Carychium* demonstrates a Holarctic distribution, *Zospeum* was known until recently only from European caves situated in the central Pyrenees, the Cantabrian Mountains, the southern, central Alps and the Dinaric Alps (Gittenberger 1980, Pezzoli 1992, Slapnik 1994, Slapnik and Ozimec 2004). However, Prozorova *et al.* (2011) have documented *Zospeum*-like shell material from the Nodong Cave in South Korea.

Based on conchological criteria, 59 carychiid morphospecies are currently thought to be valid (Bank 2012, R. Bank

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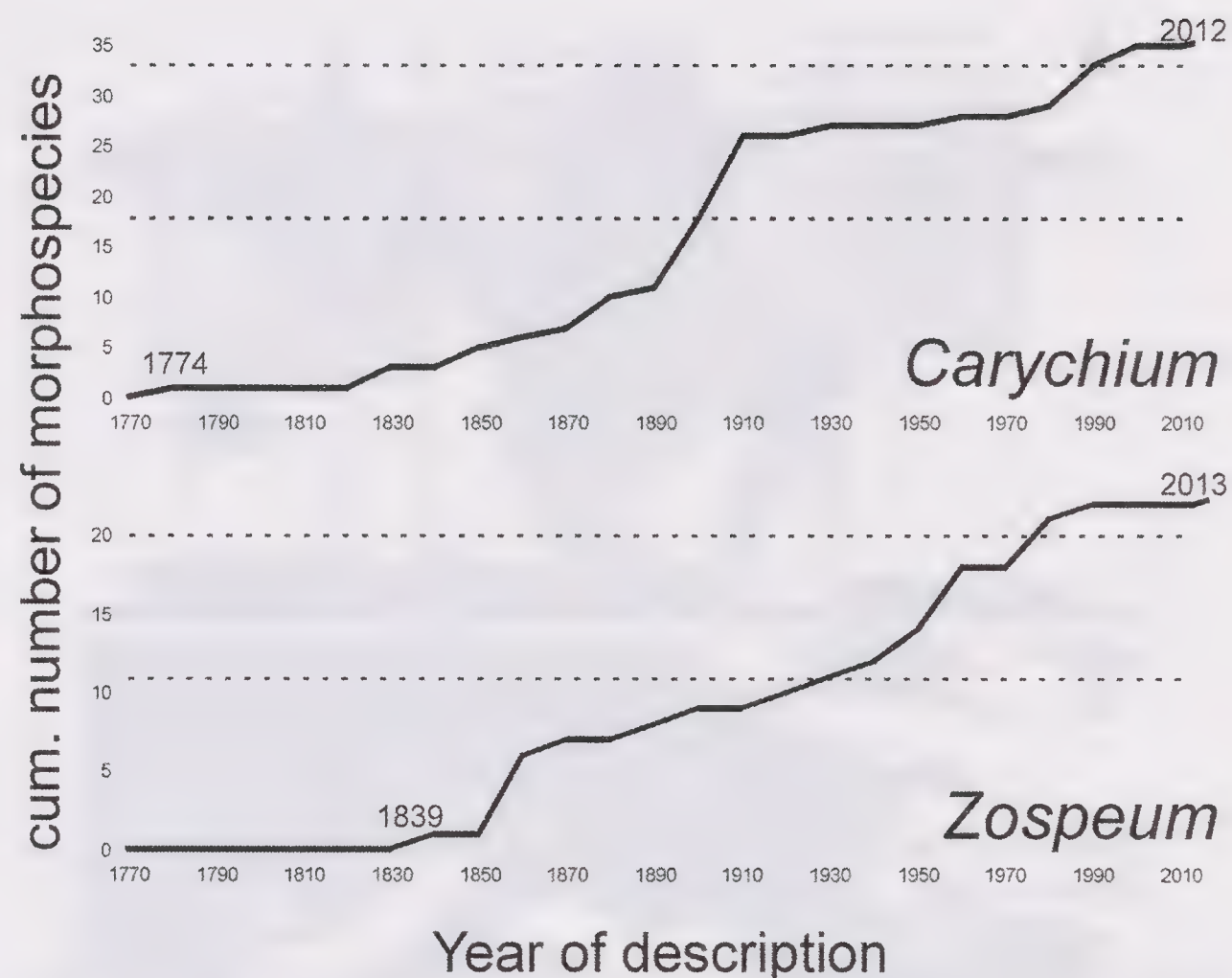


**Figure 1.** Members and habitat of Carychiidae. **A**, *Carychium* specimen. **B–C**, Typical epigeal habitats of *Carychium*. **D**, *Zospeum* specimen (image taken by J. Bedek, HBSD). **E–F**, Typical subterranean habitats of *Zospeum*. Arrows point to specimens. (Color shown in electronic version only).

and R. Slapnik pers. comm.) (Fig. 2). At the same time, the degree of synonymy (including species and subspecies designations) is more than two-thirds. Notably, the majority of synonymies is linked to the carychiid type species: *Carychium minimum* O. F. Müller, 1774 and *Zospeum spelaeum* (Rossmässler, 1839). Description ‘hot-spots’ refer to Europe, North America, and East-Asia (Fig. 3). However, these regions reflect taxonomic expeditions, subjective travel routes and interest in malacology rather than true biodiversity. Hence, the finding of two, potentially new *Carychium* species from China (Weigand *et al.* 2013) reflects the collector’s botanic itinerary rather than mirror the biodiversity of this region. Interest in subterranean biodiversity was ignited when the first cave-dwelling organism was found during the late 18<sup>th</sup> Century (the blind cave salamander, *Proteus anguinus* (Laurenti, 1768) from Slovenia) (Aljančič 2012), initiating a bonanza of discovery and frenzy amongst taxonomists to describe other cave-dwelling taxa—including *Zospeum*.

### CONTINUOUS PHENOTYPIC VARIABILITY

Varying environmental conditions may cause a shift in abundance and distribution of phenotypes for a given species or evolutionary lineage. Phenotypic variability, with overlap between morphospecies, has been demonstrated in numerous gastropod groups such as the Ampullariidae, Cochlicopidae, and Lymnaeidae (Giusti and Manganelli 1992, Estebenet and

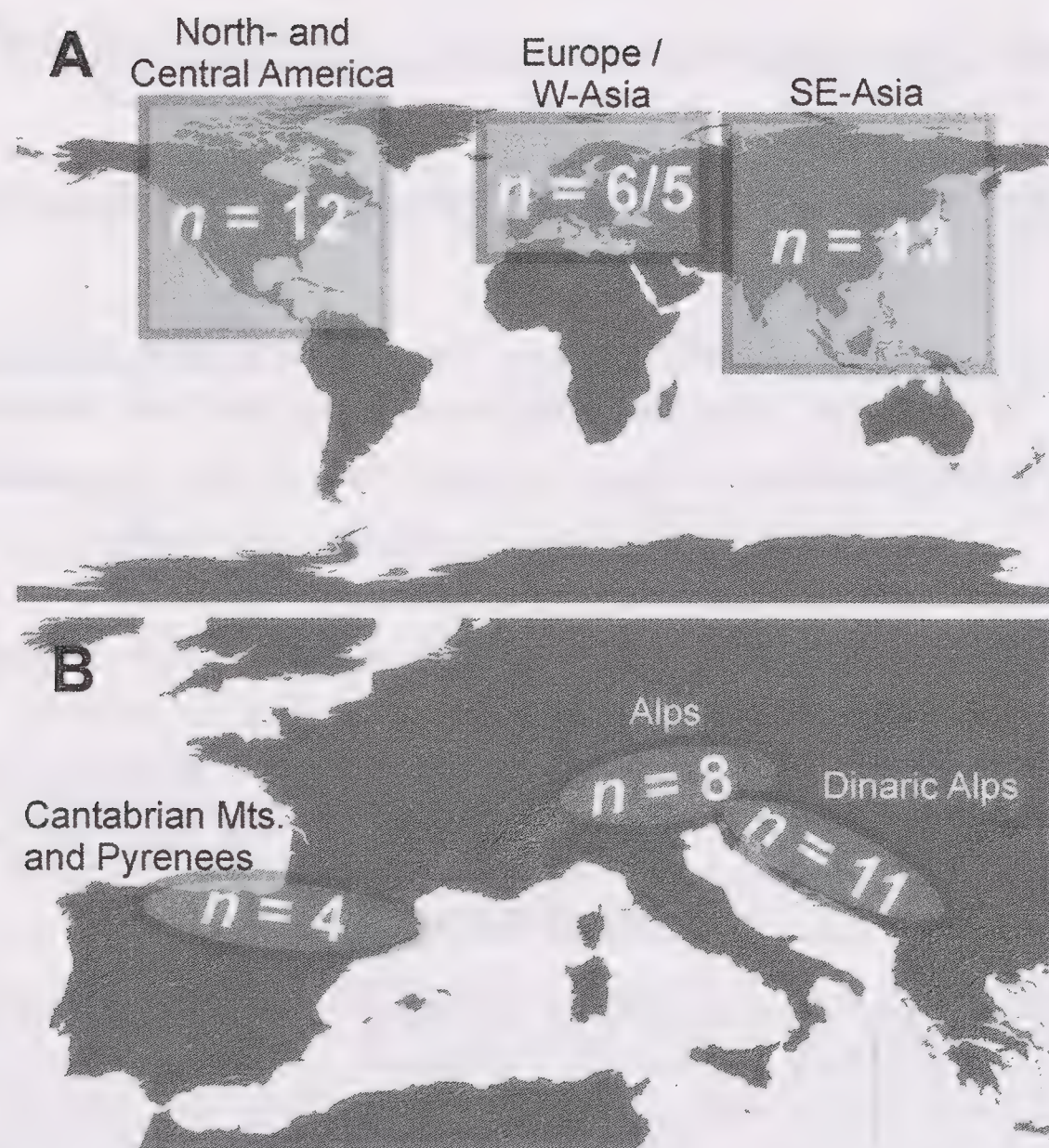


**Figure 2.** Description profiles of Carychiidae. Indicated are the cumulative numbers of currently recognized, ‘valid’ morphospecies hypotheses (y-axis) over time (x-axis). Years of the first and last description are indicated. The dashed lines refer to the 50% and 90% levels of described morphospecies.

Martín 2003, Pfenninger *et al.* 2006, Correa *et al.* 2011, Nekola *et al.* in press). While variable environmental conditions can lead to highly polymorphic lineages, stable environments maintain certain morphotypes and, thus, favor morphological stasis (Wake *et al.* 1983, Weigand *et al.* 2013). This represents a problem for taxonomic and systematic concepts based on gastropod shell characteristics; necessitating a solution to unravel inherent shell plasticity in congruence with the task of distinguishing between intraspecific variability and interspecific boundaries. Usually, a phenotype hypothesis is postulated after taxonomically assessing, sorting, comparing and evaluating a limited number of specimens. However, these formalized phenotype hypotheses only conceptualize the true diversity expressed by all possible phenotypes of a taxon, *i.e.*, the phenome of an evolutionary lineage (Deans *et al.* 2012).

In carychiid taxonomy, standard keys refer to characters such as the level of striation, shell dimensions and proportions, the form and position of the columellar fold as well as the number of whorls (Zimmermann 1925, Pilsbry 1948, Bole 1974, Strauch 1977, Burch and Van Devender 1980, Bank and Gittenberger 1985). It is not surprising that several of these conchological characteristics demonstrate a certain amount of intraspecific phenotypic variability. In particular, the analysis of large datasets of (geographically widespread) specimens provides valuable information. The comprehensive morphometric study conducted by Nekola and Barthel (2002) on North American material of *Carychium exile* H. C.





**Figure 3.** Geographic distribution of Carychiidae. The number of morphospecies ( $n$ ) per geographic region for *Carychium* (A) or mountain range for *Zospeum* (B) is indicated.

Lea, 1842 and *Carychium exiguum* (Say, 1822) observed a continuous latitudinal variation in shell heights and widths for both species. Geographical variation of shell dimensions and proportions was also illustrated in Zimmermann (1925) on a pan-European set of *Carychium*. Bulman's (1990) analysis of Polish *Carychium tridentatum* (Risso, 1826) revealed a wide variability within and between populations, linking specimens by a continuous phenotype sequence. Most populations demonstrated significant variation in at least two characters, e.g., shell dimensions, shell proportions or whorl number. Moreover, Bulman (1990) emphasized that the interpretation of form and position of the columellar fold depended on the number of whorls and the angle of view respective of the whorl number. In addition to the variable shell shape, comparative anatomical investigations of Carychiidae are complicated by their minute size and seasonal hermaphroditism (Morton 1954, Jochum 2011).

### IMPLEMENTATION OF DNA BARCODES

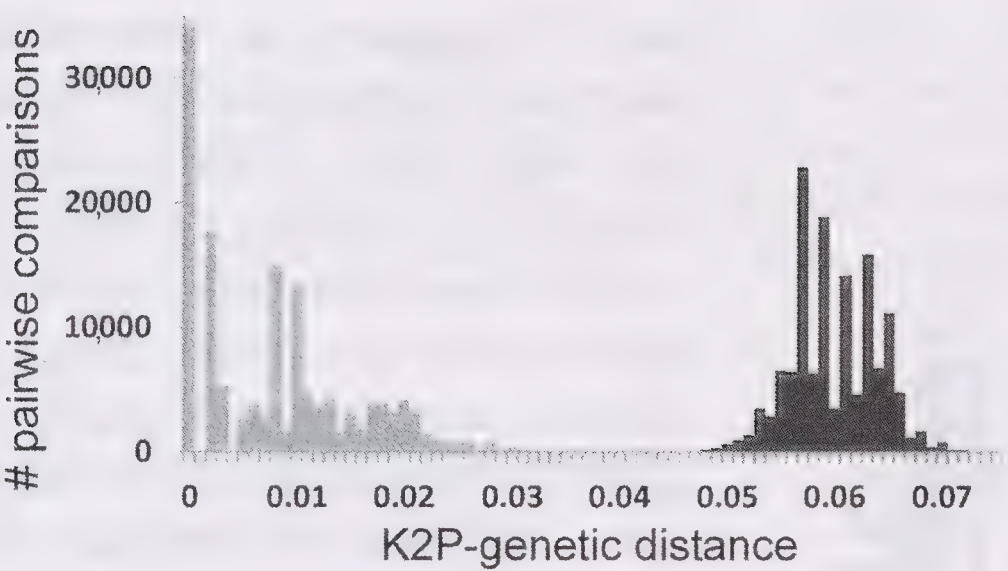
Since the 1960s, molecular data has been implemented to assist taxonomic and systematic surveys (Suárez-Díaz and Anaya-Muñoz 2008). Weigand *et al.* (2011) introduced DNA barcodes, i.e., fragments of the cytochrome c oxidase subunit I (COI) for species identification and delineation in Carychiidae.

The initial study indicated that 90% of carychiid morphospecies designations were supported by molecular data. Additionally, their investigation revealed cases of past taxonomic lumping and splitting due to high intraspecific phenotypic variability and morphological stasis. However, only a few individuals per population and morphospecies were investigated. A more comprehensive survey discussing the evolution of the Carychiidae unraveled a large number (50%) of morphologically unrecognized evolutionary lineages (Weigand *et al.* 2013). A certain discrepancy between conchologically-driven and molecular taxonomy exists (Weigand *et al.* 2011, 2013, Weigand, Goetze *et al.* 2012).

### RE-EVALUATION OF CONCHOLOGICAL CHARACTERS OF THE OUTER SHELL – THE SETUP

By using DNA-barcoded specimens in a retrospective approach, we explored the intraspecific variability of shell characteristics within two European *Carychium* species: *Carychium minimum* (CM) and *C. tridentatum* (CT). Specimens were retrieved from a larger phylogeographical study (Weigand, Pfenninger *et al.* 2012). An unambiguous genetic delineation was possible for all 742 specimens (CM: 325, CT: 417) in more than 270,000 pairwise comparisons (Fig. 4). Within and between species, genetic variability is separated by a barcoding gap of 3.4–4.3% Kimura-2-parameter (K2P)-distance. After molecular identification, the objective was to clarify the utility of a tractable strategy to augment common taxonomic practice within Carychiidae: the weighing of shell dimensions (shell height 'H', shell width 'W', length of the last whorl 'LW' and length of the upper whorls 'UW') and shell proportions (shell length to shell width 'L/W' and the last whorl to the upper whorls 'LW/UW'). Specimens were measured (CM = 161; CT = 110) by counting pixels in images (456 pixels = 1.00 mm) between measurement landmarks (Fig. 5). Additionally, shell proportions were calculated. Uni- and bivariate biostatistical analyses of morphometric data for the investigated characters were conducted in GraphPad Prism version 4.03 (San Diego, California, U.S.A.). Both datasets were tested for a Gaussian distribution using the D'Agostino-Pearson test (D'Agostino and Stephens 1986). Subsequently, and due to the fact that both datasets were not normally distributed, the Mann–Whitney–U–test for continuous, unpaired samples was conducted (Mann and Whitney 1947). Box-and-whisker diagrams were applied for visualization of numerical data (Benjamini 1988). The upper and lower whiskers indicate the sample maximum and minimum, respectively. Boxes are restricted by the 25<sup>th</sup> (lower boundary) and 75<sup>th</sup> percentiles (upper boundary), including the median.

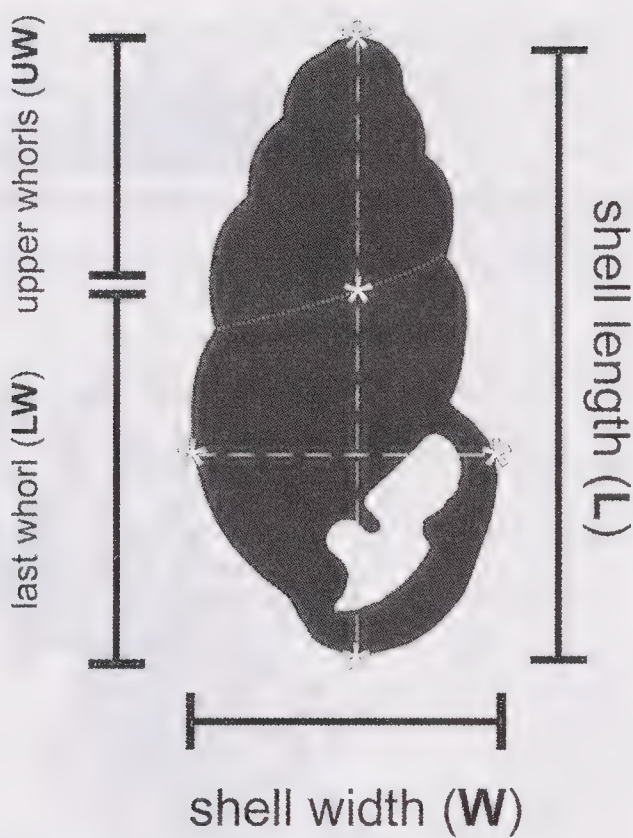




**Figure 4.** Genetic delimitation. The frequency distribution of the pairwise comparisons for 742 DNA barcoded individuals of *C. minimum* and *C. tridentatum* is shown. Intraspecific (grey) and interspecific (black) genetic variability (as Kimura-2-paramter, K2P-distance) are non-overlapping and separated by a barcoding gap.

**RE-EVALUATION OF CONCHOLOGICAL  
CHARACTERS OF THE OUTER SHELL – FINDINGS  
AND CONSEQUENCES**

Summarized in Table 1 are the mean values ( $\pm$  SD) of metric shell characters for *CM* and *CT* obtained from this study’s genetically-identified material and empirical data extracted from carychiid literature (Zimmermann 1925, Watson and Verdcourt 1953, Bulman 1990). Shell dimensions and proportions demonstrated continuous variability, often indicating a considerable degree of interspecific overlap (Fig. 6). We emphasize that these areas represent defined zones of overlap. Whereas, in the case of potential mtDNA introgression events (Currat *et al.* 2008), one would expect the occurrence of randomly distributed values across the morphospaces of both species. Error-rates, here defined as incongruencies between the molecular identification and a conchological assignment, were inferred by a canonical discriminant analysis conducted in SPSS version 12.0 (IBM, New York). DNA barcode assignments were considered as the fixed variable. At least 8 % of incongruently identified individuals (including all shell characteristics) were revealed for



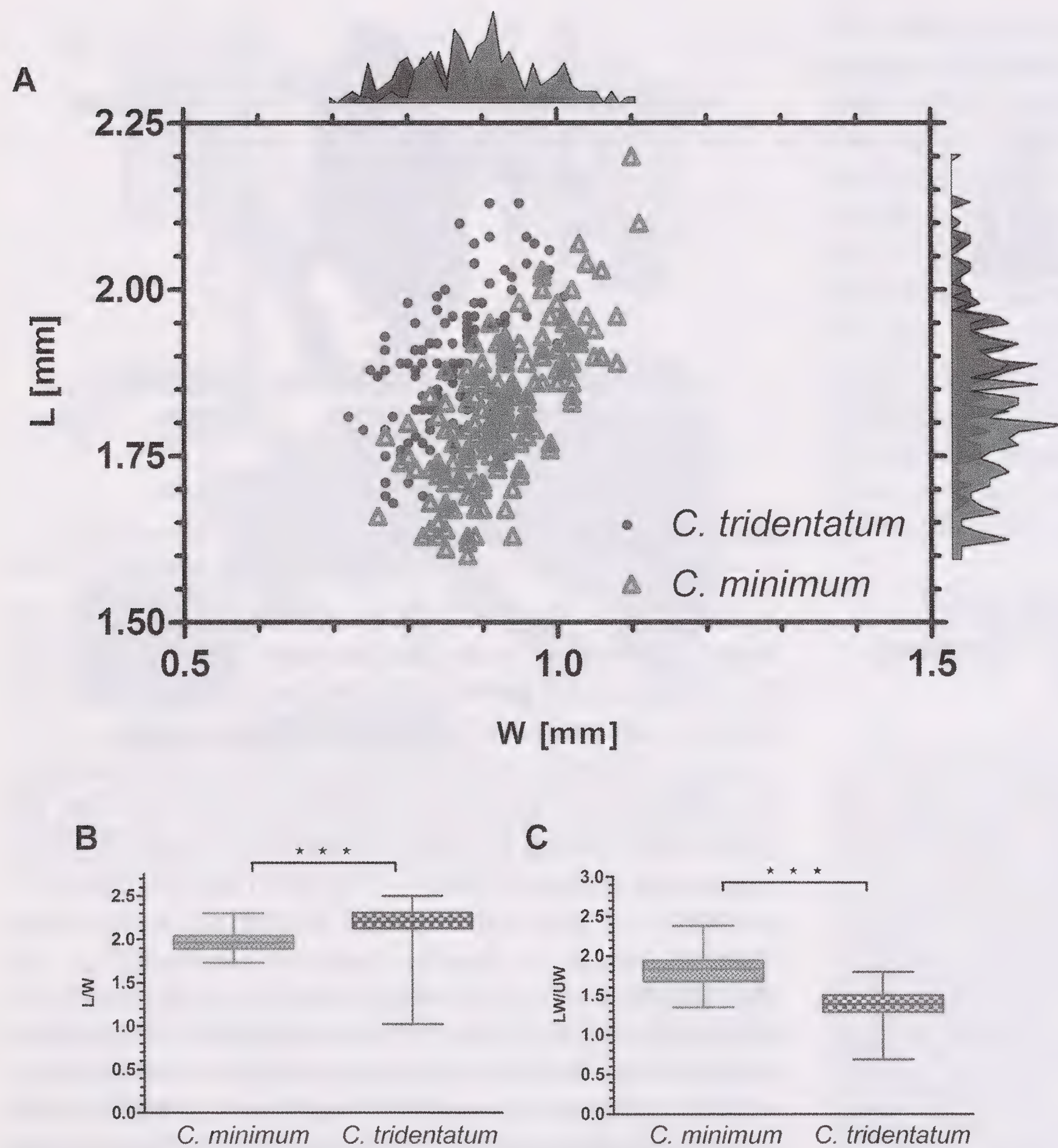
**Figure 5.** Measurement landmarks.

a classification. An identification procedure relying on shell length only resulted in the maximal error-rate of 34 % (Table 2). In general, the power of discrimination was higher with shell proportions than shell dimensions. This suggests a characteristic allometric shell shape for both taxa for which absolute changes in a given shell dimension are buffered by the proportional change of another dimension (Shingleton 2010). Hence, the allometric shape of characteristic phenotypes is sufficient for qualitative species identification (presence/absence), which is already intuitively performed by the differentiation of a more robust *CM* and a more slender *CT* phenotype (Fig. 7). If a quantitative assessment or acquisition of knowledge about the population structure at localities of sympatry is the primary aim, the complementary application of molecular data is requisite. As demonstrated, DNA barcodes of *CM* and *CT* are non-overlapping and clearly discriminate between intra- and interspecific genetic variability (Fig. 4). We deposited DNA barcodes of Carychiidae in the Barcode Of Life Data system (BOLD; <http://www.boldsystems.org>) (Ratnasingham and Hebert 2007) in the projects ‘Phylogeography of *Carychium*’ (PHYCA) and ‘Barcoding carychiid microsnails’ (BARCA).

**Table 1.** Mean values for shell dimensions [in mm  $\pm$  SD] and ratios [ $\pm$  SD] of *C. minimum* and *C. tridentatum* specimens. Mean values of morphometric measurements extracted from relevant literature are given for I: Zimmermann (1925); II: Watson and Verdcourt (1953) and III: Bulman (1990). L: shell length; W: shell width; LW: last whorl; UW: upper whorls.

| Shell characters | <i>Carychium minimum</i> |      |      | <i>Carychium tridentatum</i> |      |      |      |
|------------------|--------------------------|------|------|------------------------------|------|------|------|
|                  | own data                 | I    | II   | own data                     | I    | II   | III  |
| L                | 1.81 $\pm$ 0.11          | 1.80 | 1.79 | 1.89 $\pm$ 0.10              | 1.95 | 1.95 | 1.74 |
| W                | 0.92 $\pm$ 0.07          | 0.95 | 0.87 | 0.87 $\pm$ 0.12              | 0.93 | 0.84 | 0.83 |
| L/W              | 1.97 $\pm$ 0.11          | 1.90 | 2.06 | 2.20 $\pm$ 0.17              | 2.10 | 2.32 | 2.09 |
| LW               | 1.17 $\pm$ 0.07          |      |      | 1.09 $\pm$ 0.07              |      |      |      |
| UW               | 0.65 $\pm$ 0.07          |      |      | 0.80 $\pm$ 0.08              |      |      |      |
| LW/UW            | 1.81 $\pm$ 0.20          |      |      | 1.38 $\pm$ 0.16              |      |      |      |





**Figure 6.** Morphometry. Results of the conchological measurements for *Carychium tridentatum* (blue) and *C. minimum* (red) are given. **A**, Overlapping distributions of shell length (L) and shell width (W). **B**, Box-and-whisker plot for the ratio shell length/width. Both species demonstrate significantly different means. However, all variability of *C. minimum* lies within the conchological range of *C. tridentatum*. **C**, Box-and-whisker plot for the ratio last whorl/upper whorls. Both species demonstrate significantly different means. However, both species possess overlapping distributions. (Color shown in electronic version only).

Contrary to the variable *Carychium* shell, the *Zospeum* shell demonstrates a rather limited spectrum of phenotypic variability. Since the more stable environmental conditions encountered in subterranean habitats select and stabilize for an evolved phenotypic adaptive optimum, cave populations are known to be prone to convergent evolution of phenotypes and commonly feature limited variability (Juan *et al.* 2010). Exemplary is the case of Dinaric *Zospeum*, including seven populations collected at type localities (Fig. 8). DNA barcoding revealed the two morphospecies, *Zospeum isselianum* Pollonera, 1887 and *Zospeum spelaeum schmidtii* (Frauenfeld, 1854) to comprise more than one distinct, and so far, morphologically unrecognized evolutionary lineage

each (Weigand *et al.* 2011, 2013). In particular, geographically widespread taxa were found as valid proxies in predicting the existence of unrecognized evolutionary lineages in *Zospeum*. Since these species are already listed as vulnerable or endangered (Ozimec *et al.* 2009), knowledge about the existence of (cave-) endemic evolutionary lineages instead of a widespread taxon is of highest conservation value. Pitfalls of DNA barcoding for the delineation of carychiid species arise when incomplete lineage sorting or introgression after hybridization are present (Moritz and Cicero 2004). We regard both as valid risks in cases where two or more morphospecies share (or have very closely related) DNA barcodes. Moreover, the existence of clearly, genetically separated evolutionary lineages (*i.e.*, having distinct DNA barcodes and nuclear DNA) is best explained by the differentiation of isolated populations irrespective of accompanying morphological, ecological or behavioral changes (de Queiroz 2007).

Intraspecific variability of shell characteristics (high for *Carychium*, low for *Zospeum*) was sometimes misinterpreted when postulating former morphospecies hypotheses in Carychiidae. A fact that Burch and Van Devender (1980) in their latest revision of North American *Carychium* acknowledged as follows: “[...] some authors [...] have not treated *C. exile* and *C. exile canadense* as being distinct from *C. exiguum*. Similar

problems may exist in the *C. mexicanum*, *C. floridanum* and southern *C. exile* forms”. In particular, assumedly widespread and (moderately) variable morphospecies seem to be affected, *e.g.*, *Carychium pessimum* Pilsbry, 1901, *Carychium noduliferum* Reinhardt, 1877, *Carychium mexicanum* Pilsbry, 1891, *Carychium costaricanum* Von Martens, 1898, *Zospeum suarezi* Gittenberger, 1980, *Zospeum spelaeum schmidtii* (Frauenfeld, 1854), and *Zospeum isselianum* Pollonera, 1887 (Weigand *et al.* 2013). In light of addressing the question of magnitude – the known and the unknown – *i.e.*, how many cryptic species we might expect in respect to our own studies, the situation can be best explained by the morphospecies, *Z. isselianum*. It can be estimated, via information gleaned from the literature (Italian, Austrian, Slovenian, Croatian, and



**Table 2.** Results of the Canonical Discriminant Analysis. A prior grouping of DNA barcoded specimens (CM: *Carychium minimum*, CT: *C. tridentatum*) was tested given different conchological classifications. Provided are the numbers of congruently ('CM as CM' and 'CT as CT') as well as incongruently classified specimens ('CM as CT' and 'CT as CM'). Percent values are shown in brackets. For each variable, the total congruence (T), *i.e.*, original grouping correctly classified, is provided in percent. Canonical correlation = 0.807; Eigenvalue = 1.872; Wilk's Lambda = 0.348;  $\chi^2 = 287.984$ ;  $p = 0$ ;  $df = 4$ . Prior assignment probabilities were calculated from individual group sizes.

| Variable(s)                              | CM as CM   | CT as CT   | CM as CT  | CT as CM  | T (%) |
|------------------------------------------|------------|------------|-----------|-----------|-------|
| all variables ('L'+ 'W'+ 'L/W'+ 'LW/UW') | 158 (94.6) | 98 (89.1)  | 9 (5.4)   | 12 (10.9) | 92.4  |
| shell proportions ('L/W'+ 'LW/UW')       | 158 (94.6) | 97 (88.2)  | 9 (5.4)   | 13 (11.8) | 92.1  |
| last whorl/upper whorls ('LW/UW')        | 149 (89.2) | 100 (90.9) | 18 (10.8) | 10 (9.1)  | 89.9  |
| shell length/shell width ('L/W')         | 153 (91.6) | 86 (78.2)  | 14 (8.4)  | 24 (21.8) | 86.3  |
| shell dimensions ('L'+ 'W')              | 149 (89.2) | 88 (80.0)  | 18 (10.8) | 22 (20.0) | 85.6  |
| shell width ('W')                        | 133 (79.6) | 63 (57.3)  | 34 (20.4) | 47 (42.7) | 70.8  |
| shell length ('L')                       | 138 (82.6) | 46 (41.8)  | 29 (17.4) | 64 (58.2) | 66.4  |

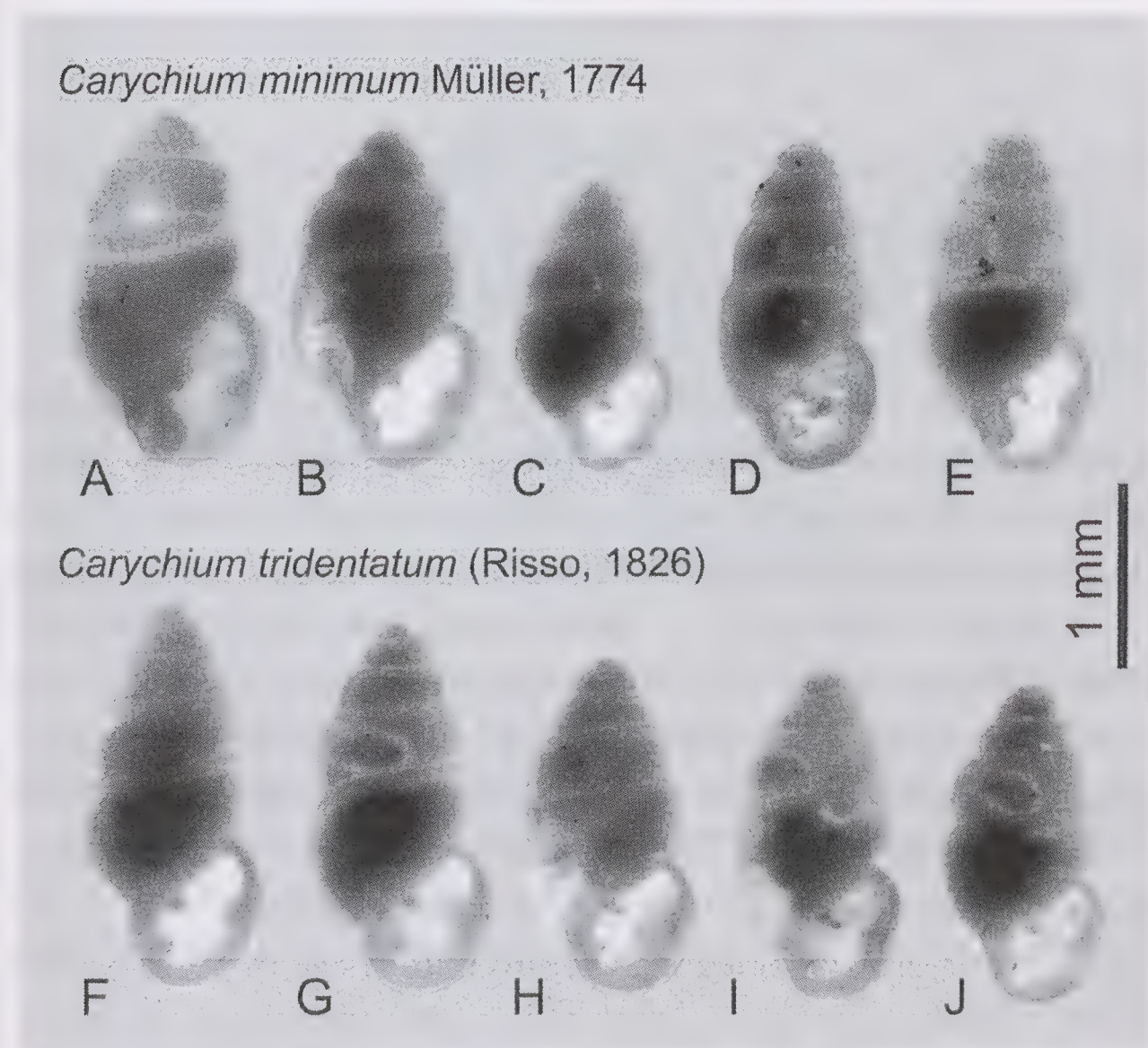
Bosnia Herzegovinian finds) and museum collections housed by the Slovenian Academy of Sciences and Arts in Ljubljana, the Slovenian Museum of Natural History, and the Croatian Biospeleological Society Collection in Zagreb (R. Slapnik, unpublished data), that we are dealing with over 300 localities for this morphospecies alone. If taken one step further, and the 8,000 plus caves in Croatia (Bedeck *et al.* 2006) are considered for potential finds of *Zospeum* populations, we soon find we are juggling immense numbers of isolated populations and potentially, morphologically unrecognized evolutionary lineages. We emphasize the sheer magnitude of *Zospeum* biodiversity in all caves of their potential distribution. Since they

harbor valuable intraspecific genetic resources, resources that might vanish even if other populations of the species survive (Bálint *et al.* 2011), conservation of these subterranean systems is paramount.

#### FUTURE DIRECTIVE TOWARDS INTEGRATIVE EVIDENCE

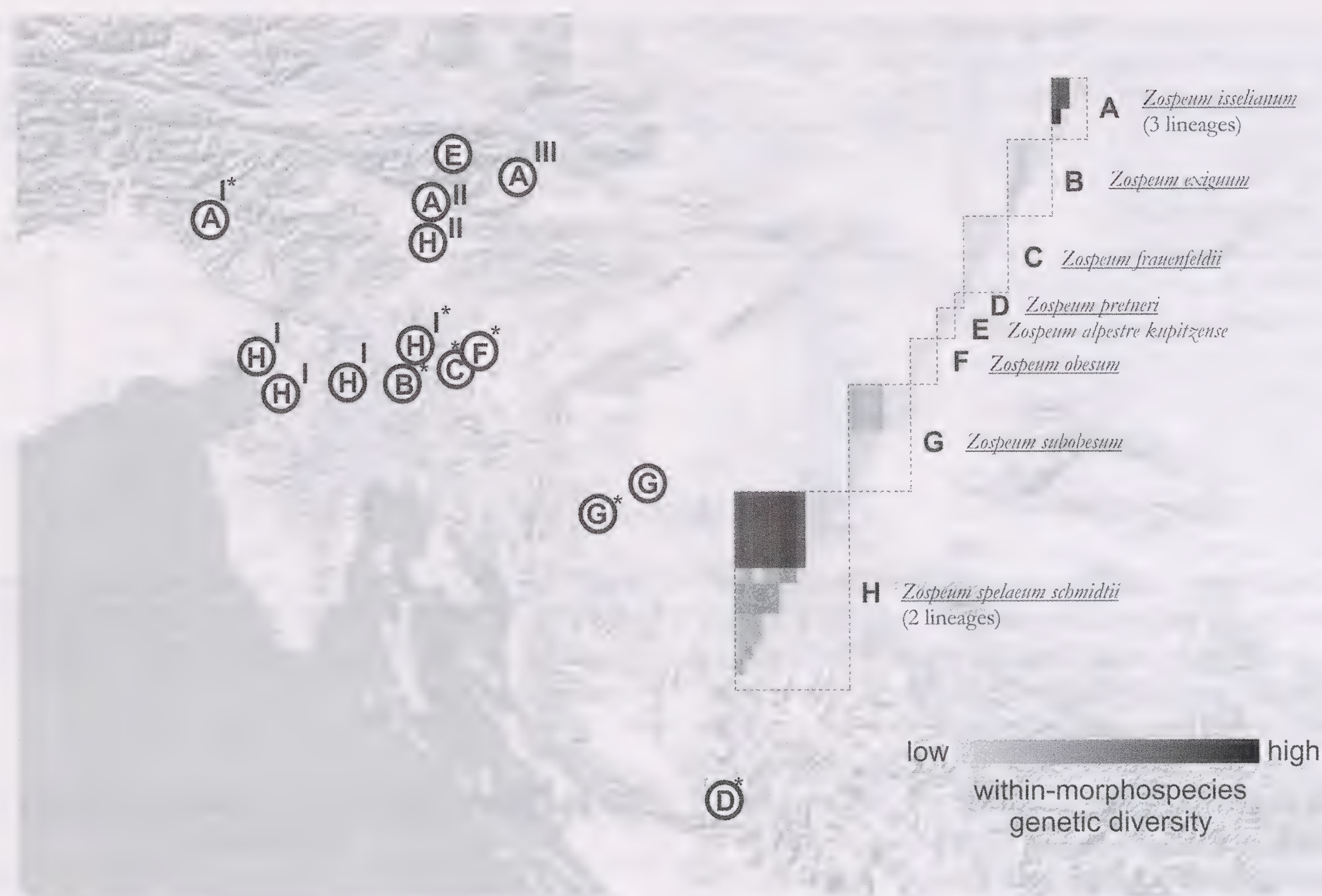
Taxonomic names are often the only link between different data sources. The informative value for all taxonomic characteristics is high when character states are well separated and easy to identify. If taxonomy is ambiguous or wrong, downstream analyses may be considerably affected. Taxonomic errors greatly affect interpretation of a) the richness, distribution and abundance of taxa, b) the taxonomic age estimates retrieved from the fossil record, c) the reconstruction of paleovegetation types based on ecological requirements of gastropod species, d) bioclimatic niche models constructed from digital occurrence data, and e) outcomes of monitoring and conservation studies and lastly, management and protection strategies of species.

We strongly encourage employing multiple lines of evidence in postulating species hypotheses. Ambiguous (morphological) taxonomic identification is not confined to a certain group and many taxonomists are faced with similar issues (*e.g.*, Porco *et al.* 2012). For this reason, an integrative taxonomic approach is needed. In addition to other considerations, the following points can help taxonomists to avoid ambiguities associated with investigations of single characteristics: 1) Integration of multiple datasets (*e.g.*, morphology, ecology, genetics, geography, reproductive isolation), 2) collection and (morphological + molecular) characterization of type locality populations to link historic morphospecies hypotheses and genetic data, 3) non-invasive DNA isolation to preserve voucher specimen shells (*e.g.*, by foot tissue clipping (Haskell and Pan 2010) or as performed in Weigand (2013)), 4)



**Figure 7.** Intraspecific variability of *Carychium* phenotypes. The most characteristic (to the left) and intermediate phenotypes (to the right) are illustrated for the two species *C. minimum* (upper row) and *C. tridentatum* (lower row).





**Figure 8.** Dinaric *Zospeum*. Geographic distribution and genetic delimitation of Dinaric *Zospeum* morphospecies (modified after Weigand *et al.* 2011, 2013). Evolutionary lineages, including specimens from the type locality, are underlined. Type localities are designated by an asterisk. The heat-map indicates intra-morphospecies genetic diversity (light grey: low; black: high). Distinct cave populations of the morphospecies *Z. spelaenum schmidtii* and *Z. isselianum* are also genetically identified as separate evolutionary lineages (I–III).

analyses in conjunction with the examination of type specimens, and 5) deposition of data sources to ensure repeatability (e.g., voucher specimens in museum collections, DNA barcodes in online databases, biological datasets in Dryad or phylogenetic tree hypotheses in TreeBase). The “cyber-centipede” *Eupolybothrus cavernicolus* Komerički & Stoev, 2013 may serve as an example (Stoev *et al.* 2013).

Currently, the authors are working on an integrated revision of the Carychiidae, investigating specimens from type localities, museum material including types, and implementing morphological and molecular datasets as well as a new imaging technology (nanoCT scanning) (see Jochum *et al.* 2013). Specifically, we are focusing on the columellar fold, since this character has long been established as a diagnostic trait in carychiid taxonomy (Watson and Verdcourt 1953, Lozek 1957, Strauch 1977, Burch and Van Devender 1980, Bank and Gittenberger 1985). However, its information content must be re-evaluated (see e.g., Bulman 1990) by using different datasets.

In conclusion, the Carychiidae were not spared the species lumping and splitting tendencies of earlier taxonomists.

Notably, apparently widespread morphospecies, fossil taxa and descriptions of often highly endemic (sub-) species may be greatly affected, resulting in potential, long-term identification failures (Villa and Villa 1841, Pilsbry 1891, 1894, Clapp 1906, Zimmermann 1925, Strauch 1977). In one case, Pilsbry (1948) stated that the “two specific names introduced by Bourguignat seem to have been based upon selected large (*existelium*, length 2 ½ mm), and small (*euphaeum*, length 1 ¾ mm) specimens [...] Both were described as smooth, and without doubt are synonymous with *C. exiguum*”. In addition, the astonishing discovery of nine *Carychium* species (including two first descriptions) at a single fossil site in Poland (Stworzewicz 1999) could be interpreted as an alternate or successive occurrence of distinguishable states of a few evolutionary lineages. Although all these problems have been widely reported, naturalists, conchologists and malacologists are still churning up new species without due consideration of today’s multifaceted advances in taxonomy, as in the case of *Carychium arboreum* Dourson, 2012 (“The shell is 1.8–2.1 mm in height, pupa-shaped [...]”) (Dourson 2012). The integration of molecular data along with its potentiality



to flag cases of cryptic species further enables taxonomists to better evaluate the degree of information derived from traditionally-applied conchological characters. Moreover, after cryptic species are flagged, in-depth conchological investigations may well reveal so far overlooked, diagnostic characteristics congruent with the molecular delimitation, rendering the species pseudo-cryptic. On the other hand, it should be emphasized that much valuable knowledge about the distribution, ecology and species diversity of carychiids has been gleaned from amateur scientists. In the final analysis, only a cooperative, integrative approach will enable us to know what we treasure and what we are losing in the long run.

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